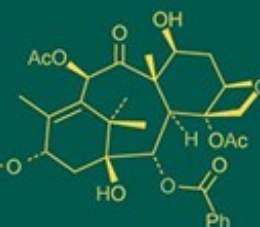
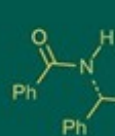
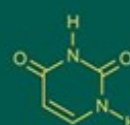


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Screening of chickpea (*Cicer arietinum* L.) genotypes *Pusa JG-16* and *PDKV Kabuli-4* for drought tolerance under PEG 6000-induced osmotic stress at the seedling stage

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ABSTRACT

Background: Water deficit is a major constraint to chickpea establishment and productivity, particularly under rainfed production conditions. Controlled osmotic-stress assays provide a useful preliminary approach for identifying genotypic differences during germination and early seedling development.

Methods: Two chickpea genotypes, *Pusa JG-16* and *PDKV Kabuli-4*, were evaluated under control conditions and 10% polyethylene glycol 6000 (PEG 6000)-induced osmotic stress in a completely randomized factorial experiment with three replications. Germination percentage was recorded on the fourth day after treatment, whereas shoot and root lengths were measured on the twelfth day. Root-to-shoot ratio was calculated from corresponding replication-wise root and shoot measurements. The data were subjected to factorial analysis of variance.

Results: Under control conditions, *Pusa JG-16* and *PDKV Kabuli-4* recorded 98.30 and 96.47% germination, respectively. Under 10% PEG 6000, germination declined to 95.30% in *Pusa JG-16* and 53.53% in *PDKV Kabuli-4*. Shoot length decreased from 7.77 to 5.20 cm in *Pusa JG-16* and from 3.97 to 2.57 cm in *PDKV Kabuli-4*. Root length was largely maintained in *Pusa JG-16*, changing from 25.43 to 25.37 cm, whereas *PDKV Kabuli-4* showed a numerical increase from 10.17 to 11.33 cm. The root-to-shoot ratio increased from 3.28 to 4.88 in *Pusa JG-16* and from 2.57 to 4.45 in *PDKV Kabuli-4*. Genotype, PEG 6000 treatment and their interaction significantly affected germination, whereas the main effect of PEG 6000 on root length was not significant.

Conclusion: *Pusa JG-16* exhibited a more favourable early-stage response to 10% PEG 6000, particularly through maintenance of germination and greater absolute shoot and root growth. The findings provide preliminary evidence of contrasting responses to controlled osmotic stress and support further evaluation under soil-based and field drought conditions.

Keywords: Chickpea, Germination, Osmotic stress, Polyethylene glycol 6000, Root-to-shoot ratio, Seedling growth

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Introduction

Chickpea (*Cicer arietinum* L.) is an important grain legume cultivated extensively in rainfed and water-limited environments. Water deficit is one of the major constraints affecting chickpea production and can adversely influence germination, seedling establishment, vegetative growth, flowering and reproductive development. The effects of water limitation are particularly important during germination and early seedling establishment because successful establishment depends on adequate water uptake, mobilization of seed reserves, cell expansion and coordinated development of roots and shoots.

Early-stage screening under controlled conditions provides an opportunity to identify differences among genotypes before undertaking more extensive soil-based or field evaluations. Polyethylene glycol (PEG) 6000 is widely used to impose controlled osmotic stress during germination because it reduces the water potential of the surrounding medium while generally remaining outside plant tissues. PEG-based assays have been used to characterize variation in chickpea germination and seedling responses under controlled water-deficit conditions (Michel *et al.*, 1973; Abderemane *et al.*, 2024) [2, 1].

Root and shoot characteristics are important components of early drought-response screening. Root development influences the capacity of seedlings to explore the available growth medium, whereas shoot develop

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ment reflects the ability of young plants to maintain above-ground growth under stress. Root-related traits have been associated with drought adaptation and productivity in chickpea, particularly under terminal water limitation (Kashiwagi *et al.*, 2006; Kashiwagi *et al.*, 2015) [3, 4]. Shoot characteristics have also been investigated as indicators associated with drought response in chickpea (Ramamoorthy *et al.*, 2016) [5].

Differences in drought response have been reported between desi and kabuli chickpea types. Farooq *et al.* (2018) [6] reported that drought-associated reductions in several germination, physiological and growth characteristics were generally greater in kabuli than in desi chickpea genotypes, although considerable variation exists within both groups. More recent research has further emphasized that drought tolerance in chickpea is a complex trait influenced by morphological, physiological and molecular characteristics (Karkee *et al.*, 2026) [7].

However, responses to PEG-induced osmotic stress are genotype-dependent and cannot be inferred solely from chickpea type. Direct evaluation of individual genotypes under a defined osmotic-stress condition is therefore useful for preliminary screening.

The present investigation was undertaken to compare *Pusa JG-16* and *PDKV Kabuli-4* under control and 10% PEG 6000-induced osmotic stress using germination percentage, shoot length, root length and root-to-shoot ratio as indicators of early-stage osmotic-stress response.

Materials and methods

2.1 Experimental conditions

The experiment was conducted under controlled laboratory conditions at the Biotechnology Centre, Department of Agricultural Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India.

2.2 Plant material

Two chickpea genotypes, *Pusa JG-16* and *PDKV Kabuli-4*, were used in the investigation. *Pusa JG-16* represented the desi chickpea type, whereas *PDKV Kabuli-4* represented the kabuli type. Seeds were se-

lected for uniformity before imposing the experimental treatments.

2.3 Seed sterilization

Seeds were surface sterilized with 1% sodium hypochlorite solution for 5 min. The seeds were subsequently exposed briefly to 70% ethanol and washed three times with sterile distilled water to remove residual sterilizing agents.

2.4 Preparation of PEG 6000 solution

Polyethylene glycol 6000 (PEG 6000; HiMedia, India) was used to impose osmotic stress. Two PEG treatments were evaluated: 0% PEG 6000, representing the control, and 10% PEG 6000, representing the osmotic-stress treatment. The 10% PEG 6000 solution was prepared by dissolving 10 g PEG 6000 in distilled water and making the final volume up to 100 mL. Approximately 15 mL of the respective treatment solution was supplied to each experimental unit.

2.5 Experimental design and growth conditions

The experiment consisted of two chickpea genotypes and two PEG 6000 treatments arranged factorially in a completely randomized design with three replications, resulting in four genotype $\times$ PEG treatment combinations.

Four seeds were placed on sterile germination paper in Petri dishes and supplied with the respective treatment solution. Petri dishes were sealed with parafilm to minimize moisture loss and maintained at 25 °C. Seeds were maintained in darkness for the first four days and subsequently exposed to a 16 h light period. Germination percentage was recorded on the fourth day after treatment, whereas shoot and root lengths were measured on the twelfth day.

2.6 Germination percentage

Germination percentage was determined on the fourth day after treatment using the following equation:

$$\text{Germination (\%)} = \left(\frac{\text{Number of germinated seeds}}{\text{Total number of seeds sown}} \right) \times 100$$

Three independent replications were maintained for each genotype $\times$ PEG treatment combination.

2.7 Measurement of seedling growth

On the twelfth day, seedlings were carefully removed from the germination medium. Shoot length was measured from the point of shoot emergence to the shoot tip, while root length was measured from the

point of root emergence to the root tip. Measurements were expressed in centimetres.

2.8 Root-to-shoot ratio

Root-to-shoot ratio was calculated separately for each replication using the following equation:

$$\text{Root-to-shoot ratio} = \frac{\text{Root length}}{\text{Shoot length}}$$

The ratio was calculated from the corresponding root and shoot measurements of each replication before obtaining the treatment mean.

2.9 Statistical analysis

The experiment was analysed as a 2×2 factorial experiment in a completely randomized design with three replications. Genotype, PEG 6000 treatment and genotype $\times$ PEG 6000 interaction were considered sources of variation. Analysis of variance was performed using the factorial model. Standard error of the mean [SE_m] and critical difference (CD) at the 5% probability level were calculated for comparison of genotype $\times$ PEG treatment means. The coefficient of variation (CV) was calculated from the residual mean square. Differences were considered statistically significant at $P \leq 0.05$.

2. Results

3.1 Germination percentage

Both genotypes exhibited high germination under control conditions. *Pusa JG-16* recorded 98.30% germination, whereas *PDKV Kabuli-4* recorded 96.47%. Exposure to 10% PEG 6000 resulted in only a small reduction in germination of *Pusa JG-16*, from 98.30 to 95.30%, corresponding to a reduction of 3.00 percentage points or 3.05%. In contrast, germination of *PDKV Kabuli-4* declined markedly from 96.47% under control conditions to 53.53% under 10% PEG 6000, representing a reduction of 42.93 percentage points or 44.51%. Consequently, *Pusa JG-16* maintained substantially higher germination under osmotic stress.

The effects of genotype, PEG 6000 treatment and genotype $\times$ PEG 6000 interaction were highly significant ($P < 0.001$). Under 10% PEG 6000, *Pusa JG-16* recorded 41.77 percentage points higher germination than *PDKV Kabuli-4*.

$SE_m \pm$: 0.760 CD at 5%: 2.478 CV (%): 1.53

Table 1. Germination percentage of chickpea genotypes under PEG 6000-induced osmotic stress

Genotype	0% PEG (%)	10% PEG (%)	Reduction (percentage points)	Reduction (%)
Pusa JG-16	98.30	95.30	3.00	3.05
PDKV Kabuli-4	96.47	53.53	42.93	44.51
Mean	97.38	74.42		

3.2 Shoot length

Shoot elongation was reduced under 10% PEG 6000 in both genotypes. Pusa JG-16 recorded a mean shoot length of 7.77 cm under control conditions, which declined to 5.20 cm under 10% PEG 6000, representing an absolute reduction of 2.57 cm or 33.05%.

In PDKV Kabuli-4, shoot length decreased from 3.97 cm under control conditions to 2.57 cm under 10% PEG 6000, corresponding to a

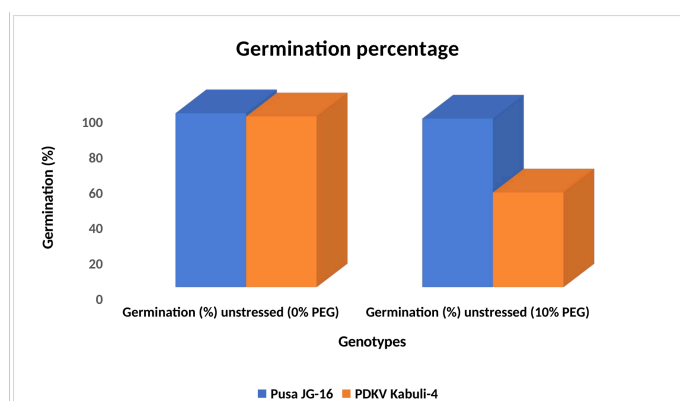
reduction of 1.40 cm or 35.29%.

Genotype and PEG 6000 treatment effects were highly significant, while the genotype $\times$ PEG 6000 interaction was also significant ($P = 0.009$). Although the proportional reductions were relatively similar, Pusa JG-16 retained substantially greater absolute shoot growth under osmotic stress.

Table 2. Shoot length of chickpea genotypes under PEG 6000-induced osmotic stress

Genotype	0% PEG (cm)	10% PEG (cm)	Reduction (cm)	Reduction (%)
Pusa JG-16	7.77	5.20	2.57	33.05
PDKV Kabuli-4	3.97	2.57	1.40	35.29
Mean	5.87	3.88		

$SE_m \pm$: 0.171 CD at 5%: 0.557 CV (%): 6.07



3.3 Root length

Root length showed a contrasting response to shoot growth. Pusa JG-16 recorded a mean root length of 25.43 cm under control conditions and 25.37 cm under 10% PEG 6000, representing a difference of only 0.07 cm or 0.26%.

PDKV Kabuli-4 recorded root lengths of 10.17 and 11.33 cm under control and 10% PEG 6000 conditions, respectively, representing a numerical increase of 1.17 cm or 11.48%.

The genotype effect on root length was highly significant ($P < 0.001$). However, the main effect of PEG 6000 treatment was not significant ($P = 0.212$), and the genotype $\times$ PEG 6000 interaction was also non-significant ($P = 0.167$). Therefore, the numerical increase in root length observed in PDKV Kabuli-4 should not be interpreted as a statistically significant stimulatory effect of PEG treatment.

Pusa JG-16 maintained substantially greater absolute root length than PDKV Kabuli-4 under both experimental conditions.

Table 3. Root length of chickpea genotypes under PEG 6000-induced osmotic stress

Genotype	0% PEG (cm)	10% PEG (cm)	Change (cm)	Change (%)
Pusa JG-16	25.43	25.37	-0.07	-0.26
PDKV Kabuli-4	10.17	11.33	+1.17	+11.48
Mean	17.80	18.35		

$SE_m \pm$: 0.406 CD at 5%: 1.322 CV (%): 3.89

3.4 Root-to-shoot ratio

The root-to-shoot ratio increased under 10% PEG 6000 in both genotypes. In Pusa JG-16, the ratio increased from 3.28 under control conditions to 4.88 under osmotic stress, representing an increase of 1.61 or 49.01%.

In PDKV Kabuli-4, the ratio increased from 2.57 to 4.45, corresponding to an increase of 1.88 or 73.20%.

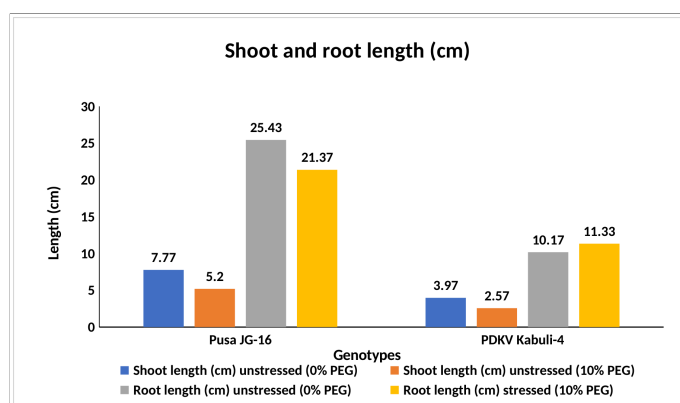
The effects of genotype and PEG 6000 treatment were significant, whereas the genotype $\times$ PEG 6000 interaction was non-significant ($P = 0.421$). The increase in root-to-shoot ratio primarily reflected greater restriction of shoot elongation relative to root development.

Table 4. Root-to-shoot ratio of chickpea genotypes under PEG 6000-induced osmotic stress

Genotype	0% PEG	10% PEG	Increase	Increase (%)
Pusa JG-16	3.28	4.88	1.61	49.01
PDKV Kabuli-4	2.57	4.45	1.88	73.20
Mean	2.92	4.67	-	-

(continued on next page)

$SE_m \pm$: 0.161 CD at 5%: 0.526 CV (%): 7.37



3.5 Comparative response under 10% PEG 6000

Under 10% PEG 6000, *Pusa JG-16* recorded higher values for all four measured characteristics than *PDKV Kabuli-4*. The greatest difference occurred in germination percentage, followed by root length and shoot length.

Table 5. Comparative performance of chickpea genotypes under 10% PEG 6000

Trait	<i>Pusa JG-16</i>	<i>PDKV Kabuli-4</i>
Germination (%)	95.30	53.53
Shoot length (cm)	5.20	2.57
Root length (cm)	25.37	11.33
Root-to-shoot ratio	4.88	4.45

Overall, *Pusa JG-16* maintained stronger germination and greater absolute seedling growth under the imposed osmotic-stress condition.

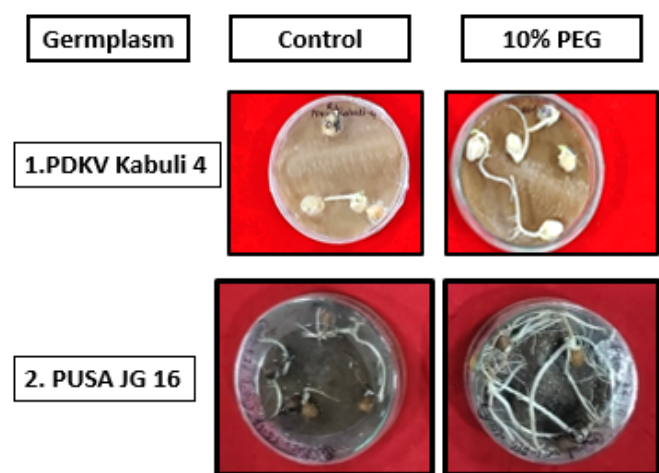


Figure 1. *In vitro* screening using discriminating dose of PEG 6000 for examining drought resistance

4. Discussion

The present study demonstrated clear differences between *Pusa JG-16* and *PDKV Kabuli-4* in their early-stage responses to PEG 6000-induced osmotic stress. The strongest separation between the genotypes was observed for germination, while differences in absolute root and shoot growth further indicated contrasting seedling performance.

Germination was particularly responsive to the imposed osmotic treatment. *Pusa JG-16* maintained 95.30% germination under 10% PEG 6000 compared with 53.53% in *PDKV Kabuli-4*. The relatively small reduction observed in *Pusa JG-16* indicates that its germination process was less affected by the imposed reduction in external water potential. In contrast, the marked reduction in *PDKV Kabuli-4* indi-

cates greater sensitivity during the germination phase. PEG-based screening has previously been shown to differentiate chickpea genotypes according to their germination and seedling responses under controlled water-deficit conditions (Abderemane *et al.*, 2024) [1].

The present findings are broadly consistent with previous observations that drought responses can differ between desi and kabuli chickpeas. Farooq *et al.* (2018) [6] reported greater drought-associated reductions in several germination, physiological and growth characteristics in kabuli genotypes than in desi genotypes. However, the present experiment involved only one desi and one kabuli genotype. Therefore, the observed contrast should be attributed to the specific genotypes evaluated rather than generalized to all desi and kabuli chickpeas.

Shoot length decreased under PEG treatment in both genotypes. *Pusa JG-16* showed a 33.05% reduction, while *PDKV Kabuli-4* showed a 35.29% reduction. Although the proportional reductions were relatively similar, *Pusa JG-16* maintained 5.20 cm shoot length under stress compared with 2.57 cm in *PDKV Kabuli-4*. Thus, assessment based solely on percentage reduction may not fully describe stress response when genotypes differ substantially in their baseline growth. Absolute growth retained under stress should also be considered when comparing seedling performance.

Root development showed a different response from shoot growth. *Pusa JG-16* maintained a root length of approximately 25.4 cm under both treatments, whereas *PDKV Kabuli-4* had considerably shorter roots. Importantly, the statistical analysis indicated that the main effect of PEG treatment on root length was not significant and that the genotype $\times$ PEG interaction was also non-significant. Consequently, the numerical increase in root length observed in *PDKV Kabuli-4* under PEG treatment should not be interpreted as a statistically established stimulatory effect of osmotic stress.

Nevertheless, the large difference in absolute root length between the two genotypes is biologically noteworthy. Root characteristics have been associated with the capacity of chickpea plants to exploit available soil water under drought conditions (Kashiwagi *et al.*, 2006; Kashiwagi *et al.*, 2015) [3, 4]. However, root length measured in a Petri-dish assay should not be equated directly with field rooting depth, root length density or field water-extraction capacity. The present assay should therefore be interpreted as an early-stage morphological screening approach rather than a direct assessment of field drought adaptation.

The increase in root-to-shoot ratio under PEG stress in both genotypes indicates that shoot elongation was more strongly restricted than root elongation. The ratio increased from 3.28 to 4.88 in *Pusa JG-16* and from 2.57 to 4.45 in *PDKV Kabuli-4*. Because the genotype $\times$ PEG interaction was non-significant, this increase should be interpreted primarily as a general response to the imposed osmotic treatment rather than as a genotype-specific interaction. Moreover, the ratio should be considered together with the underlying root and shoot measurements because a higher root-to-shoot ratio can result from enhanced root development, reduced shoot growth, or both.

Taken together, *Pusa JG-16* exhibited a more favourable early-stage phenotype under 10% PEG 6000, characterized particularly by maintenance of germination and greater absolute shoot and root growth. These observations are consistent with the broader literature showing substantial genetic variation in chickpea response to water deficit and the importance of early establishment and root-related traits in drought adaptation (Farooq *et al.*, 2018; Karkee *et al.*, 2026) [6, 7].

However, PEG-induced osmotic stress represents a simplified laboratory condition and does not reproduce all components of field drought. Field drought involves progressive soil drying, changes in soil hydraulic properties, root-soil interactions, atmospheric demand, temperature and stress duration. In addition, drought adaptation involves physiological and biochemical processes such as osmotic adjustment, antioxidant activity, stomatal regulation and maintenance of photosynthetic activity. Therefore, the present findings should be regarded as preliminary evidence of differential early-stage osmotic-stress response rather than definitive proof of field drought tolerance.

Further studies should validate the observed differences using soil-based water-deficit experiments and field evaluation and should include physiological and biochemical measurements such as relative water content, membrane stability, chlorophyll or photosynthetic characteristics, compatible solute accumulation, antioxidant enzyme activity and detailed root-system architecture.

Conclusion

The present investigation revealed distinct early-stage responses of *Pusa JG-16* and *PDKV Kabuli-4* to PEG 6000-induced osmotic stress. Under 10% PEG 6000, *Pusa JG-16* maintained 95.30% germination compared with 53.53% in *PDKV Kabuli-4* and also retained substantially greater absolute shoot and root growth. The root-to-shoot ratio increased in both genotypes, reflecting greater restriction of shoot elongation relative to root development.

Among the traits evaluated, germination percentage provided the clearest separation between the two genotypes, while absolute root and shoot growth provided additional morphological evidence of contrasting seedling responses. Overall, *Pusa JG-16* demonstrated a more favourable early-stage response to the imposed osmotic-stress condition than *PDKV Kabuli-4*. However, because the experiment was conducted under controlled PEG-induced conditions and was limited to early seedling traits, the findings should be considered preliminary. Confirmation under soil-based and field drought conditions, together with physiological and biochemical measurements, is required before making broader conclusions regarding drought tolerance.

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